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Distribution of solanesol in *Nicotiana tabacum*

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Abstract: Solanesol is an important secondary metabolite in *Nicotiana tabacum.* Distribution of solanesol in *Nicotiana tabacum* was investigated by High Performance Liquid Chromatography (HPLC) method. The quantitative distribution of solanesol in various organs and tissues of *N. tabacum* showed that solanesol content, obviously different in all organs, was 6.8, 18.3, 27.5, 45.8, and 68.0 times higher in leaves than that in the stalks, flowers, seeds, fruits and roots, respectively. The contents of solanesol in various parts of leaf, stalk and flower were determined. The content of solanesol in top leaf, middle leaf and bottom leaf gradually decreased (6.124, 5.813 and 5.687 $mg \cdot g^{-1}$, respectively) and the content of solanesol in various leaf-parts (leaf apex, leaf middle and leaf base) also gradually decreased. The content of solanesol in top stalk was 1.19 times and 1.92 times higher than that in the middle stalk and the bottom stalk, respectively. The content of solanesol in various tissues of stalk (epidermis, cortex and stele) dramatically decreased. The sepal contained higher concentration of solanesol (1.192 $mg \cdot g^{-1}$) compared to any other parts in flower. The study will provide the base data for the regulation and control of solanesol, moreover, it will provide the scientific evidences for the rational development and utilization of *N. tabacum* resources.

Keywords: Solanesol; *Nicotiana tabacum*; Distribution; High Performance Liquid Chromatography

Introduction

Nicotiana tabacum belongs to the Solanaceae family and the plant is considered to be a good source of a large number of bioactive substances. Recently, the chemical compositions of *N. tabacum* have attracted considerable attentions in the world (Svob-Troje *et al*. 1997; Crofcheck *et al*.2003; Wang *et al.* 2004; Cepeda-Benito *et al.* 2006; Zu *et al*. 2006). Solanesol, a 45-carbon, all-trans-nonaprenol (Fig. 1), was first isolated from flue-cured tobacco (Rowland *et al*. 1956). Solanesol itself can be used as antiulcer and hypertension treating agent (Kijima, *et al*. 1979; Tahara *et al*. 1980). In addition, solanesol is a necessary medical intermediate in the industrial synthesis of coenzyme Q_{10} (Rüegg *et al*. 1959; Lipshutz *et al*. 2005), which is an excellent medicine in cardiovascular disease, cancer, atherosclerosis and so on (Portakal *et al*. 2000; Rundek *et al*. 2004; Yalcin *et al*. 2004; Küttner *et al*. 2005; Weant *et al*. 2005).

In virtue of the important medical effect of soanesol, it is very important to know the distribution of the solanesol in *N. tabacum* in order to choose the right part and to obtain good resources for extraction of solanesol. Solanesol is in fact found in many plants from the Solanaceae family, one member of which is the *Nicotiana* genus. Other members of the family known to contain solanesol include tomato plants, potato plants, eggplants and pepper plants (Douce *et al*. 2001). However, it was reported that the content of solanesol in *N. tabacum* was considerably higher than that in other plants and thus this plant represented the most convenient source for large-scale isolation of solanesol (Duan *et al*. 2000). Another significant advantage of *N. tabacum* is its large biomass production, compared to most other crop plants (Daniell *et al*. 2001). By now solanesol is considered to be a ubiquitous component in leaf and the contents of solanesol in leaves of *N. tabacum* were studied by many researchers (Severson *et al*. 1977; Narosimha-Rao *et al*. 2000). In this study, a detail investigation of the distribution of solanesol in various organs and tissues of *N. tabacum* was carried out. In particular, the concentrations of solanesol in various parts, leaf-parts, stalk-parts and flower-parts of *N. tabacum* were determined and compared.

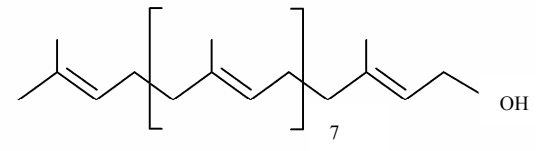

Fig. 1 Molecular structure of solanesol

Materials and methods

Plant materials

N. tabacum were collected from the arboretum in Northeast Forestry University, China. The two varieties of *N. tabacum* are K326 and NC89. Roots, stalks, leaves, flowers, seeds and fruits materials were separated from *N. tabacum* for the determination

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of solanesol contents.

The third (top), the seventh (middle) and the eleventh (bottom) leaf were picked from stalk. Furthermore, each leaf was separated into three parts (Leaf apex, leaf middle and leaf base). Top stalk (between the second and third leaf), middle stalk (between the sixth and seventh leaf) and bottom stalk (between the tenth and eleventh leaf) were excised from *N. tabacum* and each stalk was separated into three parts (epidermis, cortex and stele) with scalpel and blade. Pedicel, sepal, ovary, petal, androecium and gynoecium were excised from the mature flowers.

The above samples were air-cured, ground to pass a 60-mesh screen and stored in sealed plastic bags before analyses.

Sample preparation and extraction

The sample pretreatment process involved extraction with 85% ethanol under the frequency of 40 kHz, at a temperature of 45 °C for 15 min in an ultrasonic device (KQ 250 DB model, Kunshan ultrasonic instrument Inc., China) and with the ratio of liquor to material of 15 $mL \cdot g^{-1}$. Similar process was repeated three times for complete extraction. After sonication, the 85% ethanol extracts were combined and evaporated to dryness in vacuo. For determination of solanesol content, the concentrate was dissolved in the chromatographic mobile phase, vortexed for 20 s followed by centrifugation at 10000 rpm for 8 min. After filtering through a filter paper and a 0.45-μm membrane filter (Millipore), the clean supernatant was injected directly.

Determination of Solanesol by HPLC

Quantification of solanesol by HPLC has been described elsewhere (Li 2006). Chromatographic analysis was carried out by HIQ SIL $C_{18}V$ reversed-phase column (ø 4.6 mm×250 mm) packed with 5-μm diameter particles; the mobile phase was a mixture of acetonitrile and isopropanol (8:7, v/v). This mobile phase was filtered through a 0.45-μm membrane filter (Millipore), then deaerated ultrasonically prior to use. Solanesol was detected at 215 nm by UV detector. Flow rate and injection volume was 1.5 $mL \cdot min^{-1}$ and l0 μL, respectively. Quantification was carried out by the integration of the peak using external standard method. All chromatographic operations were carried out at ambient temperature.

Validation of quantitative method was performed with samples for five injections. The results of the five injections from the same sample at the five concentrations (0.01–0.1 $mg \cdot mL^{-1}$) showed similar retention time. The relative standard deviation proved that the accuracy and reproduction were excellent. The chromatogram of solanesol in tobacco leaves was shown in Fig. 2.

Chemicals

Solanesol standard（+90%）was purchased from Sigma (USA). Acetonitrile and isopropanol were of HPLC grade (Krackeler Scientific, Albany, USA). Ethanol was of analytical grade (Beijing Chemical Reagents Company, China).

Results and discussion

The concentrations of solanesol in different organs from two varieties of *N. tabacum* (K326 and NC 89) were determined by HPLC and the results were presented in Fig. 3.

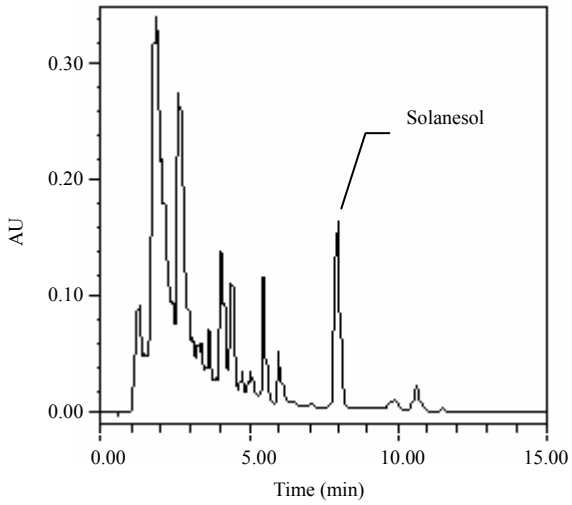

Fig.2 Chromatogram in the extract of tobacco leaves

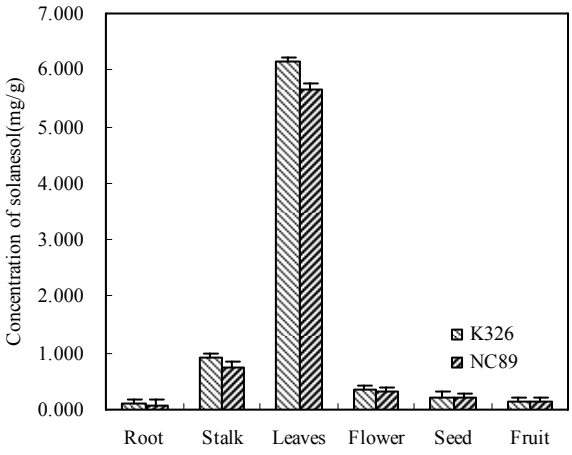

Fig. 3 Distribution of solanesol in *Nicotiana tabacum*

The results form Fig. 3 demonstrated that the contents of solanesol were significantly different from the different organs of *N. tabacum*. The maximum concentration of solanesol was obtained in leaves (5.906 mg/g), followed by the stalks and flowers (0.868 and 0.324 $mg \cdot g^{-1}$, respectively). Whereas seeds, fruits and roots only produced lower concentration of solanesol (0.215, 0.129 and 0.087 $mg \cdot g^{-1}$, respectively), the content of solanesol in leaves was 6.8, 18.3, 27.5, 45.8 and 68.0 times higher than that in the stalks, flowers, seeds, fruits and roots, respectively. The content of solanesol in *N. tabacum NC 89* followed a trend similar to that in *N. tabacum K326*.

Many literatures reported that the leaves of *N. tabacum* were the major resource of production solanesol (Masahiro *et al*. 1994; Whitfield *et al*. 2004). The above results showed that solanesol presented not only in the leaves but also in stalks, flowers, seeds, fruits and roots of *N. tabacum*. Besides the leaves, the stalks and flowers also contained the higher concentration of solanesol.

Table 1 indicated that the leaves were rich in solanesol. The contents of solanesol in various parts of leaves were not signifi-

cant different. The content of solanesol in top leaf, middle leaf and bottom leaf gradually decreased (6.124, 5.813 and 5.687 mg·g^{-1}, respectively) and the content of solanesol in various leaf-parts (Leaf apex, leaf middle and leaf base) also gradually decreased (Fig. 4).

Table 1. Contents of solanesol in various organs and tissues of *Nicotiana tobacum*

Samples	Solanesol contents (mg·g^{-1})	RSD (%) [c]
(A) Leaves [a]		
Top leaf		
Leaf apex	6.353	0.48
Leaf middle	6.087	0.34
Leaf base	5.982	0.63
Whole leaf	6.124	0.42
Middle leaf		
Leaf apex	6.171	0.35
Leaf middle	5.906	0.29
Leaf base	5.784	0.42
Whole leaf	5.813	0.31
Bottom leaf		
Leaf apex	5.982	0.55
Leaf middle	5.649	0.22
Leaf base	5.447	0.34
Whole leaf	5.687	0.29
(B) Stalks [b]		
Top stalk		
Epidermis	1.918	0.36
Cortex	1.353	0.57
Stele	0.452	0.32
Total	1.184	0.30
Middle stalk		
Epidermis	1.410	0.48
Cortex	1.025	0.55
Stele	0.321	0.41
Total	0.992	0.37
Bottom stalk		
Epidermis	0.955	0.30
Cortex	0.718	0.41
Stele	0.162	0.36
Total	0.614	0.24
(C)Flowers		
Pedicel	0.167	0.37
Sepal	1.192	0.25
Ovary	0.072	0.38
Petal	0.294	0.44
Androecium	0.201	0.56
Gynoecium	0.093	0.47
Whole flower	0.336	0.28

Notes: [a] Top leaf ---- the third leaf; middle leaf----the seventh leaf; bottom leaf----the eleventh leaf, in Fig. 4. [b] Top stalk----stalk between the second and third leaf; middle stalk---- stalk between the sixth and seventh leaf; bottom stalk----stalk between the tenth and eleventh leaf, in Fig.4. [c] RSD---- relative standard deviation (n=3）

The contents of solanesol in stalks and flowers were also analyzed. For stalks, there were obviously different contents of solanesol in various parts of stalk. The content of solanesol in top stalk, middle stalk and bottom stalk dramatically decreased (1.184, 0.992 and 0.614 mg·g^{-1}, respectively) and the maximum concentration of solanesol in stalk was observed in the top position, which is 1.19 times higher than that in the middle position and 1.92 times higher than that in the bottom position. The content of solanesol in various tissues of stalk (epidermis, cortex and stele) sharply decreased (Fig. 4).

Concerning flowers, the contents of solanesol in different parts of *N. tabacum* flower were significantly different. Only a very low content of solanesol was detected in the pedicel, ovary, petal, androecium and gynoecium. Compared to other parts in flowers, the content of solanesol in the sepal (1.192 mg·g^{-1}) was higher.

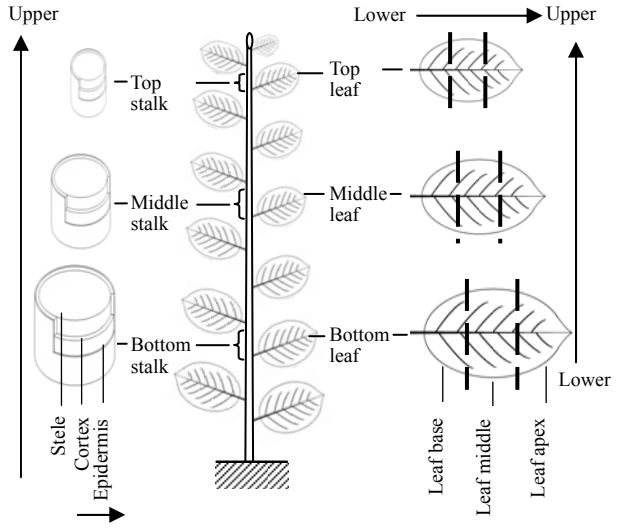

Fig. 4 Schematic picture of distribution of solanesol in leaves and stalks of *Nicotiana tabacum*

Conclusions

This study confirmed that the content of solanesol in leaves was higher than that in any other organs and the interesting result was that stalks and flowers of *N. tabacum* also contained the higher concentration of solanesol. In previous study, solanesol was mainly produced from the leaves of *N. tabacum*. The stalks of *N. tabacum* were wasted. Although the present study shows that the stalks and flowers are both good resources for production of solanesol, the biomass production of stalk is very large, compared to flowers. Thereby, the stalks, especially the top stalks of *N. tabacum* are worthy to be developed to be the new resource for production of solanesol and should be also widely used. Finally, the leaves and stalks of *N. tabacum* should be chosen as the resources for production of solanesol and the development of leaves and stalks of *N. tabacum* will play an important role in the increase of the availability of *N. tabacum*.

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